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Removal of color, COD and lignin from Pulp and Paper Mill Effluent by *Phanerochaete chrysosporium* and *Aspergillus fumigatus*

A. K. Chopra and Pushpendra Pal Singh*

Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar-249404 (Uttarakhand), India

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ABSTRACT

A study was conducted for aerobic treatment in an indegenously designed Bench-top Bioreactor to find out the degradability of color, COD and lignin content from Pulp and Paper mill effluent (PPME) using biosorption process. The Strains of Phanerochaete chrysosporium MTCC No. 787 and Aspergillus fumigatus MTCC No. 3377 procured from Institute of Microbial Technology (IMTECH), Chandigarh was used. The strains exhibited significant reduction in color, COD and lignin content of the PPME to the extent of 86%, 56% and 71% respectively with P. chrysosporium and 80%, 51% and 63% respectively with A. fumigatus after 7 days of aerobic treatment in comparison to control. The reduction in these parameters started occurring from first day of the treatment, but the maximum reduction in these parameters was observed after 7 days, at pH (5.6±0.2), temperature (25±1°C) and biomass (5% v/v) of the fungal strains. The removal with P. chrysosporium was more in comparison to A. fumigatus. The kinetic study for the rate of removal of color, COD and lignin content by both species were found to best fit a pseudo first order reaction. The rate constant was found to be inversely proportional to the concentration of parameters. The Bench-top bioreactor used for the treatment of effluent was found to be cost effective. Significant reduction in color, COD and lignin content were achieved in our bioreactor at a fraction of the cost of commercially available bioreactors making our bioreactor more effective and economical for effluent treatment by the Pulp and paper industry.

Keywords: *Aspergillus fumigatus*, Biosorption, Color, Lignin, *Phanerochaete chrysosporium.*

INTRODUCTION

Pulp and paper industry is one of the core industrial sectors in India which ranks 15th among the paper producing countries of the world. At present, there are 666 pulp and paper mills in India including 632 agro-residue and recycled fiber based units, with a manufacturing capacity of 7.6 million tons. However paper and pulp industry is highly energy intensive, consuming large amounts of fresh water, nearly 75% to 95% of which is discharged by the industries as effluent containing organic and inorganic pollutants, including coloring materials [1]. It is sixth largest polluting industry [2] of India.

The quality and quantity of processed water change depends on the methods of production. The huge amounts of wastewater discharged by the Pulp and paper mills requires elaborate effluent treatment process, which is a drain on the economy and tough on environment. The effluent generated at the pulping stage, called black liquor, contains various compounds like dissolved lignin and their degradation products, hemicelluloses, resin acid, fatty acids,

tannins and phenols. These organic compounds are also responsible for the characteristic dark brown color and toxicity of effluent. Thus, it is obligatory to treat the effluent prior to its discharge into receiving water bodies [3,4,5,6].

Since the advent of paper industry serious attempts have been made to remove this dark color of the effluent [7,8,9]. However the technique of chemical oxidation by [10,11], while precipitation methods by [12] used for removing this color are tedious and cumbersome and result in additional environmental load.

Biological methods for color removal are particularly attractive. Since lignin and its derivatives, the major contributors of black color are biodegradable [13] and have been investigated using several groups of microorganisms. These methods have potential, since they also reduce chemical oxygen demand (COD) as well as biological oxygen demand (BOD) [14]. These bio-methods involve use of fungi, bacteria, algae and enzymes as a single step treatment or in combination with other physical and chemical methods. However, the biological treatment studies have largely confined themselves to the evaluation of various microorganisms, [15]. Among the different kind of microorganisms used, white rot fungi have proved their potential in the lignin/phenolic wastewater treatment and have proved as ideal organism for decolorization as well as for the reduction of absorbable organic halides and the COD [16].

The present study was conducted to find out potential of two fungal strains *Phanerochaete chrysosporium* and *Aspergillus fumigatus* for the removal of color, COD and lignin content of Pulp and paper mill effluent using an indigenously designed Bench-top Bioreactor.

EXPERIMENTAL SECTION

Procurement and maintenance of fungal strains

Fungal strains (*P. chrysosporium* MTCC No. 787 and *A. fumigatus* MTCC No. 3377) were procured from Microbial Type Culture Collection Centre, IMTECH, Chandigarh. The fungal strains were enriched in Sabouraud broth for 3 to 7 days. Further sub culturing of fungal strains were performed from the stock culture for maintaining the strains and incubated at 5.6±0.2 pH and 25±1°C temperature for 3 to 7 days. These strains were then transferred to the Bioreactor.

Description of the fungal strains

P. chrysosporium belongs to kingdom fungi, phylum basidiomycota, class basidiomycetes and genus *Phanerochaete*. These fungi act as plant pathogens and their genus includes white rot fungus because of its specialized ability to degrade the abundant aromatic lignin polymer, while leaving the white cellulose practically untouched. The fungus releases extracellular enzymes to break-up the complex three-dimensional structure of lignin into components that can be utilized by its metabolism. It is able to degrade complex compounds such as starch, cellulose, pectin, lignin and hemicelluloses etc. [17].

A. fumigatus belongs to kingdom fungi, Phylum Ascomycota, class Eurotiomycetes and genus *Aspergillus* is a cosmopolitan and thermotolerant fungus which is isolated primarily from compost, plant material and soil. It plays an essential role in carbon and nitrogen recycling which was an important factor for decolorization process of the industrial effluent [18].

Collection of effluent from Pulp and paper mill industry

The Pulp and Paper mill effluent (PPME) was collected from the discharge point of the Star Pulp and Paper Mill Ltd. It is situated about 5 km from the Saharanpur city (Uttar Pradesh, India) which produces paper as its main product from agrobased residues. The PPME was brought to the laboratory and stored in refrigerator at 4°C.

Bioreactor design and biosorption of the effluent

The biosorption of PPME was carried out in an indegenously designed Bench–top bioreactor (Fig.1), which is consisted of glass (Borosil) aspirator bottle (volume 10.0 litre, radius 9.15 cm and height 38.0 cm) as the reaction vessel, fitted with an air tight rubber cork having six ports connected with sterilized hollow tubes of different heights. Height of the tubings used for air outlet, sample inlet for injecting sample in the reactor, inoculums inlet for culture transfer and for air outlet was maintained above the surface of the effluents in the reactor, whereas heights of the tubings used for (a) to bubble sterilized air in the effluent (b) to insert a thermostat for recording the temperature,

and (c) the addition of acid/ alkali for adjustment of pH, was maintained below the surface of the effluent and preferably about 5 cm from the bottom of the bioreactor. The lower side opening of the vessel was used for collecting samples when desired. The reaction vessel was filled with 7.0 liter effluent, inoculated with selected fungal strains (5% v/v) and mouth of the glass vessel was properly sealed with air tight rubber cork. Initially, the pH and temperature in the reactor vessel were set at 5.6 and 25°C with addition of acid/ alkali buffer solution and thermostat respectively, these values were maintained during the experiment because most of the fungus prefer acidic pH and the temperature range of 25°C - 30°C for their growth.

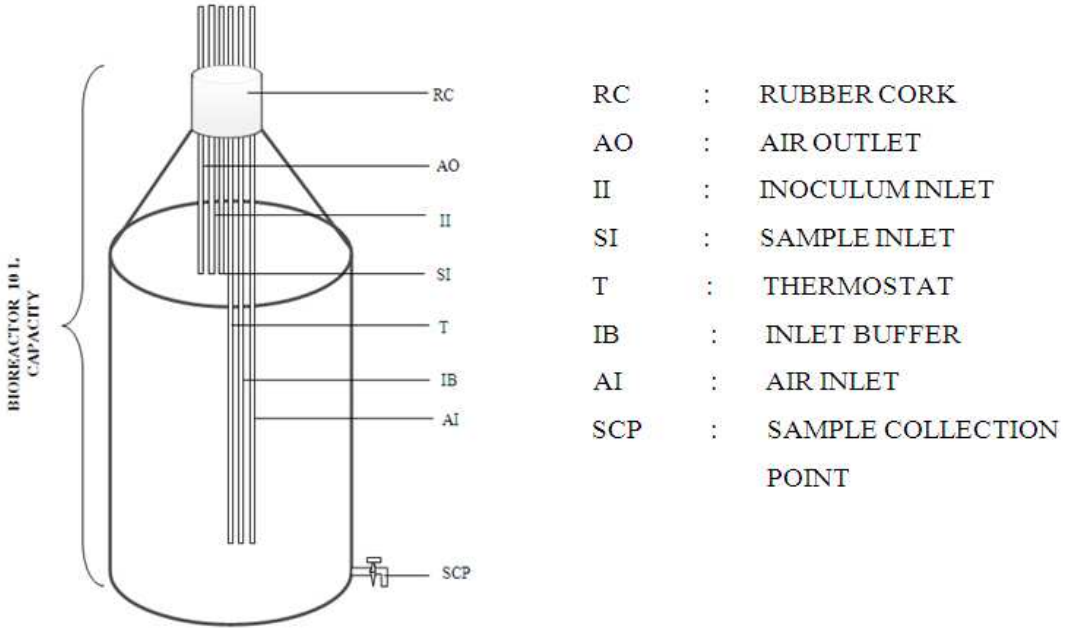


Figure1. Bioreactor design

Analytical methods

Color content in the effluent was analyzed by the method of [13]. Briefly, in this method 20 ml sample was centrifuged at 10,000 rev/min for 30 min (Model no. REMI-R-24). And the pH was adjusted to 7.6 with addition of acid/ alkali buffer solution. The absorbance was measured at 465 nm using spectrophotometer (Electronics India, Model no. 1305) and transformed into color units Platinum-Cobalt standard with a range of 0–500 color units (PCU). Lignin content and COD (Open reflux method) of the effluent were measured by the methods as given in [19].

Statistical analysis

Standard deviation (SD) and One way analysis of variance (ANOVA) were used for data analysis to measure the variations of color, COD and lignin from PPME before and after treatment using MS Excel, 2003.

RESULTS AND DISCUSSION

Characterization of PPME

The mean±SD values of physico-chemical characterization of PPME used for bioremediation showed that it contained COD 2149.67±14.50 (mg/l), lignin 6252.92±21.19 (mg/l), color 2934.75±20.62 (platinum-cobalt units) and had a pH 8.1±0.2.

Reduction in color of PPME

The significance of incubation time ranging from 4 to 21 days for the removal of color has been shown by many researchers. [20] used aerobic packed bench scale reactor with the white-rot fungus, *Trametes versicolor*, immobilized into small cubes of holm oak wood, they achieved 69% color removal from pulp mill effluent at incubation temperature of 25ºC in 2 to 5 days of incubation period. [6] obtained 78.6% color removal from pulp and

paper mill effluent by three fungal strains viz. *Merulius aureus*, an unidentified *Basidiomycete* and *Fusarium sambucinum* at pH 4.3 and temperature 30°C after 4 days of incubation time , [21] achieved 83% color removal by *P. Chrysosporium* in 4 days at pH 6.72 and temperature 32°C, where as [22] reported 84% color removal by *P. Chrysosporium* in 5 days of incubation period at pH 7.0 and temperature 30°C. The color removal after a long incubation period (21 days) was obtained by [23] at incubation temperature of 27±1°C. The percentage color removal on treatment of Pulp and paper mill effluent was observed by *P. chrysosporium* (67.78%). [24] recorded maximum reduction in color (26%) from activated sludge-treated pulp mill effluent and stated that a pH range 4-8 and temperature 20°C were more suitable for color removal by NaOH treated *Aspergilus niger* in 48 hr of incubation time.

During the present study, using Bench top bioreactor (aerobic treatment system), significant reduction in color was obtained with *P. chrysosporium* (86.28%) and *A. fumigatus* (79.67%) fungal strain in comparison to control (Effluent without strain) after 7 days of aerobic treatment at a pH (5.6±0.2) and temperature (25±1°C) (Fig. 2). The maximum reduction (86.28%) in color of PPME was recorded with *P. chrysosporium* as compared to control. However, the color removal during 7 days of treatment is encouraging. The color reduction was recorded to be slow at the early stage of aerobic treatment which increased to its maximum on the 7th day (Fig. 2) before decreasing again. This may be due to the formation of other compounds (tri-, tetra and pentachlorophenol, chlorinated catechols, chlorinated guaicols and dioxins) which are of particular importance, as they are known to be highly recalcitrant and responsible for color in the bioreactor [25]. Thus, seven days of aerobic treatment provided better nourishment in the media for the full growth and better adaptation of fungal strains.

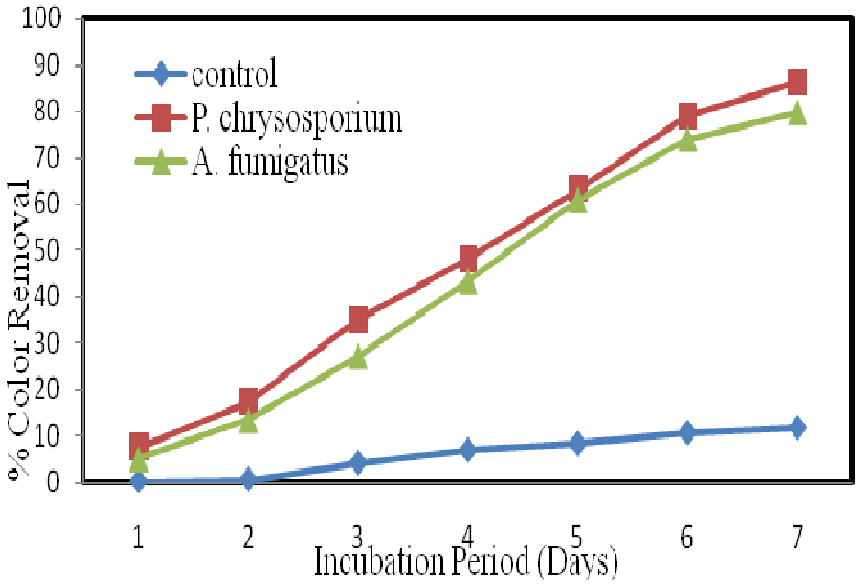


Figure 2. Percentage color removal by fungal strains at the
(pH 5.6±0.2, Temperature 25±1°C and biomass v/v 5%)

Reduction in COD

Paper mill effluent characteristically contains high BOD, COD, color and suspended particles [23,26]. The high value of COD and BOD indicates highly biodegradable nature of the effluent and thus suitable for degradation by fungi [23]. [27] observed 48% COD removal after 18 days at 38°C temperature and pH 4.5. The higher reduction in COD (89.4%) from Pulp and paper mill effluent in 4 days of aerobic treatment at temperature 30°C and pH 4.3, by the consortium (*Merulius aureus*, an unidentified *Basidiomycete*, and *Fusariums ambucinum)* immobilized on nylon mesh in a continous aereated bench-top bioreactor has been shown by [6]. [16] used *Paecilomyces sp.* (10% w/v) for aerobic treatment of Pulp and paper mill effluent in a two step bioreactor and observed 93% COD removal after 7 days of experiment at pH 8.0 and temperature 25°C. [17] achieved best reduction in COD (98.5%) from bagasse effluent by *P. chrysosporium* (biomass concentration 552 mg/l) at a temperature of 35°C and pH 6.0 after 9 days of treatment. [28], showed significant reduction in COD (78.4%) by *P. chrysosporium* from pulp mill effluent at pH 3.9, temperature 30°C on the 10th day of the experiment.

In the present study, significant reduction in COD was achieved by *P. chrysosporium* (56.32%) and *A. fumigatus* (50.82%) at a pH 5.6±0.2 and temperature 25±1°C in comparison to control. The maximum reduction in COD (56.32%) was achieved by *P. chrysosporium* (Fig. 3). The preliminary reduction in COD was slow at the early stage of treatment, it increased with increase in incubation time and maximum reduction was observed after 7 days of aerobic treatment with *P. chrysosporium*.

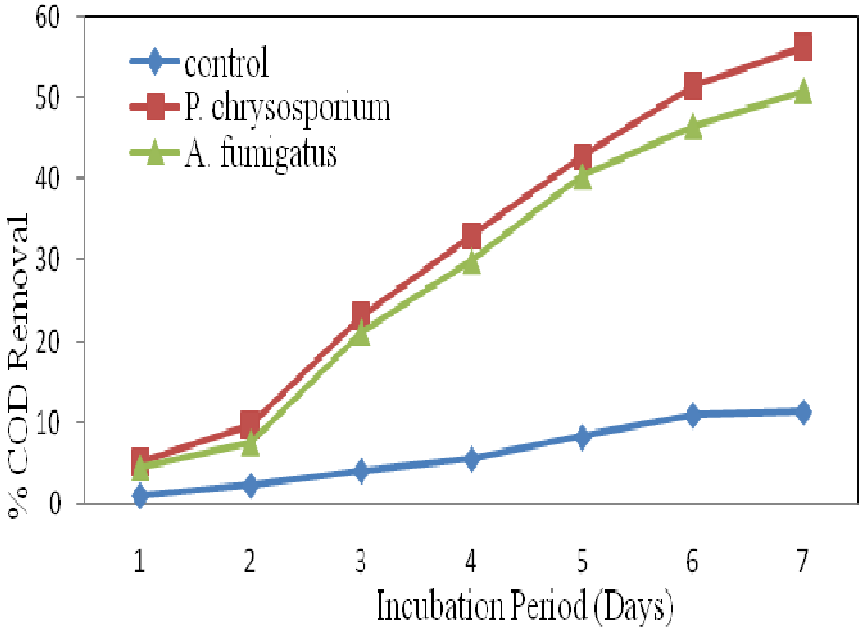


Figure 3. Percentage COD removal by fungal strains at the
(pH 5.6±0.2, Temperature 25±1°C and biomass v/v 5%) .

Reduction in lignin content

It has been observed that lignin decomposes into simple sugar and starch in bio-treatment process and then assimilated by fungus [29,30]. [31] reported best condition for lignin removal (78%) from pulp mill wastewater at pH 9.0, temperature 28°C after 16 days of biotreatment with *P. chrysosporium*. [32] reported pH 4.5 was optimum for enzyme production and lignin degradation by *P. chrysosporium*, while a pH of 5.0 was more appropriate for *Oxysporus* Sp. and *Schizophyllum commune*. The first extracellular enzyme 'ligninase', discovered to depolymerizes lignin and lignin-sub structured compounds *in vitro* were produced by this organism. A second class of enzyme also produced by *P. chrysosporium* is 'manganese peroxidase', this enzyme is known as manganese peroxidases (MnP). Manganese is known to catalyze several oxidation reactions important in lignin degradation, including decarboxylation and demethoxylation of aromatic substrates. [17] reported 70% reduction in lignin after 5 days of incubation time at pH 6.0 and temperature 30°C by *P. Chrysosporium* from bagasse effluent. In the other study of bio-treatment of pulp and paper mill effluent, [6] showed significant reduction (79.9%) in lignin by *Merulius aureus*, an unidentified *Basidiomycete* and *Fusariums ambucinum* in bench top bioreactor at pH 4.3 and temperature 30°C after 4 days of incubation time. [23] recorded reduction in lignin (63.8%) from industrial effluent after 21 days of the fungal treatment at an incubation temperature of 27±1°C using *P. chrysosporium*.

In the present study, a significant reduction was obtained in lignin content from pulp and paper mill effluent after 7 days of aerobic treatment with *P. chrysosporium* (70.52%) and *A. fumigatus* (62.91%) at a pH 5.6±0.2 and temperature 25±1°C. Maximum removal (70.52%) in lignin content was shown by *P. chrysosporium.* A continuous increase in the reduction of lignin content was observed during 7 days of incubation period (Fig. 4).

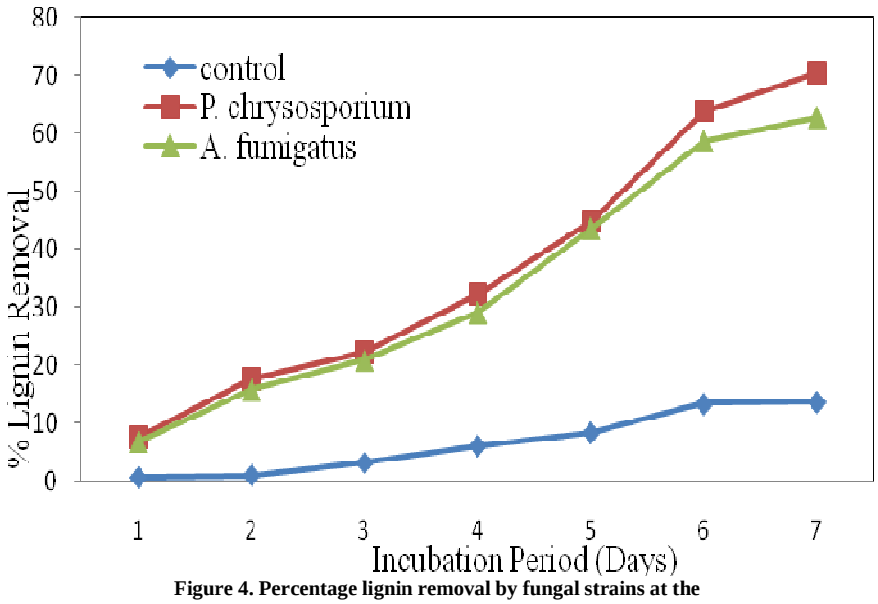


Figure 4. Percentage lignin removal by fungal strains at the
(pH 5.6±0.2, Temperature 25±1°C and biomass v/v 5%) .

Kinetic study of color, COD and lignin content

The rate of removal of color, COD and lignin content were studied and it can be represented by the first order reaction as follows [33]:

$$\ln\left(\frac{C_0}{C_t}\right) = kt$$

C_0 = Initial concentration parameter,
C_t = Concentration of parameter after t time,
k = Rate constant,
t = Time in hours.

The rate of removal of parameters (color, COD and lignin content) was studied by plotting graph between $\ln(C_0/C_t)$ vs t(day) to yield a straight line with a slope k. The straight line showed (Fig. 5) good fit of pseudo first order Kinetic model for removal of parameters by both of the fungal strains. The rate constant for removal of parameters for both fungal strains increased with a decrease in the concentration of parameters. The graphs were plotted between log k vs log C where log k value represents the rate constant and log C represents the concentration of pollutant after time t (Fig. 6 and 7). This may be due to increase in biosorption process which may increase the fungal growth and more reduction in color, COD and lignin content in seven days of the incubation period.

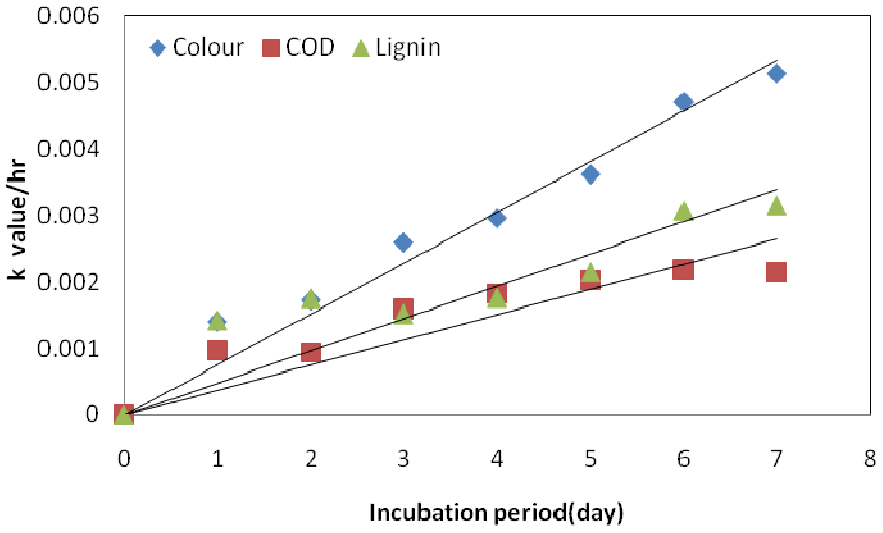
The ANOVA analysis on the data showed that decrease in the values of color, COD and lignin content were found to be affected significantly ($P < 0.001$) in comparison to control on 7th day of treatment with both *P. chrysosporium* and *A. fumigatus.*

Cost effectiveness of indegenously designed Bioreactor

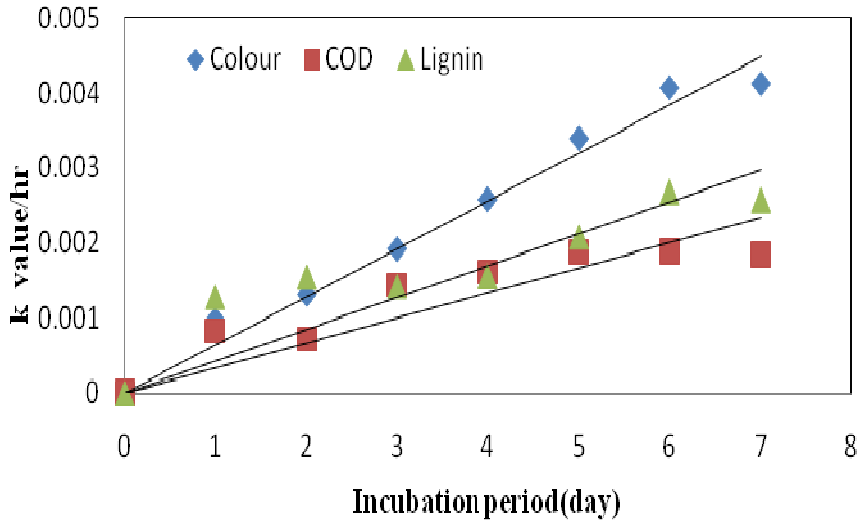
In developing countries like India, medium scale industries like Pulp and paper mill industries have a large contribution in polluting the water bodies. It affects the rate of photosynthetic reactions of plants submerged under the water body and its transparency due to its color. Installation of bioreactors for biological treatment reduces the cost of chemical treatment and provides better efficiency. A number of bioreactors with a cost from $4,500-20,000 are available in market, which have good capacity (1–200 L) for treatment of PPME. The main drawbacks of these bioreactors are high manufacturing and operational cost (Table-1) associated with their installation, which renders them uneconomical for middle scale industry.

In the present study, the cost of bioreactor, manufactured from locally available indigenous material, was very low ($165) in comparison to the cost of other bioreactors available in market (Table-2). Aeration, homogenous mixing in

bioreactor was performed by high speed aeration. Cheap glass wool was used instead of micro filters which reduced the cost.



(a)



(b)

Figure 5. (a) log C_0/C_t vs. t (day) for *P.chrysosporium*, (b) log C_0/C_t vs. t (day) for *A.fumigatus.*

Table1. Cost of commercially available Bioreactor

Bioreactor type	Capacity (Litre)	Cost in USD ($)	Manufacturing company
Fermentor bioreactor	14.0 L	10,000	Bioforce Co., Ltd. (South Korea)
Laboratory fermentor	5.0 L	15,000	Shanghai Gaoji Biological Engineering Co., Ltd. (China)
Laboratory fermentor/ bioreactor	1.0 L	4,500	Bio-Age Equipment and Services (India)
BIOF-Fermentor	5.0 L	20,000	Shanghai Gaoji Biological Engineering Co., Ltd. (China)
Stainless steel bioreactor	200L	15,000	Shanghai Ritai Medicine Equipment Project Co., Ltd. (China)

Source: www.alibaba.com

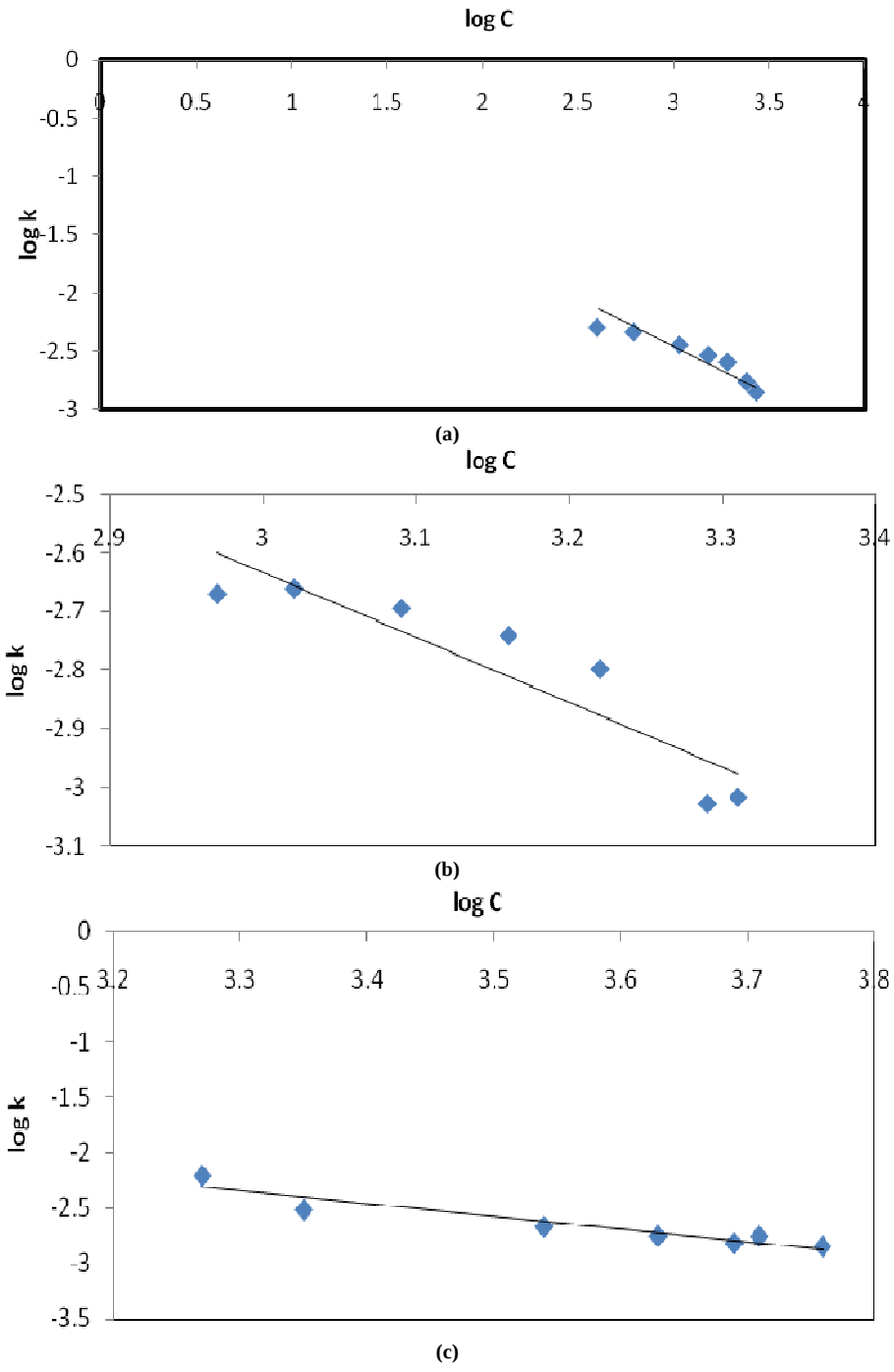


Figure 6. log k vs log C (a) Color (b) COD (c) Lignin for *P. Chrysosporium.*

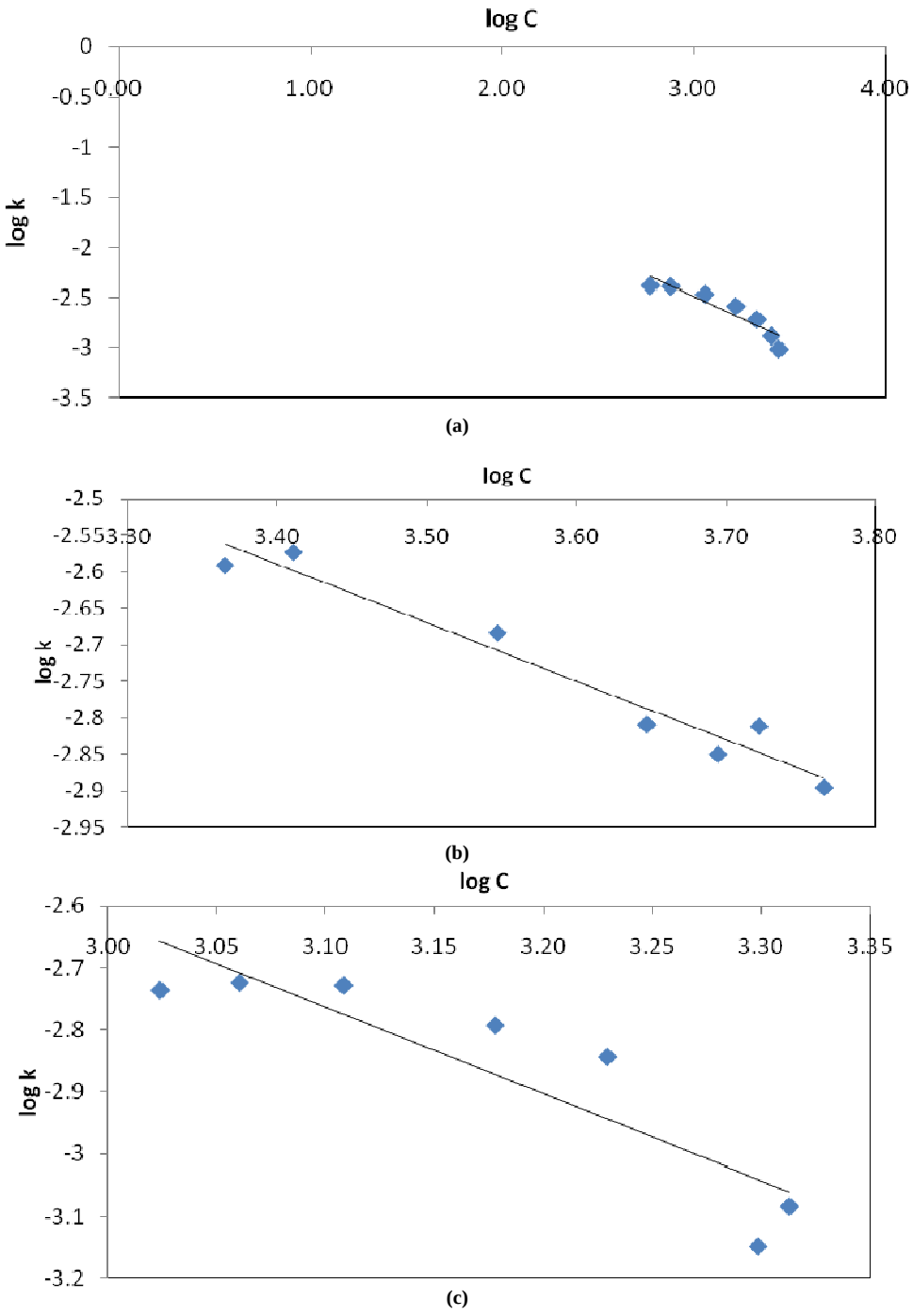


Figure 7. log k vs log C (a) Color (b) COD (c) Lignin for *A. fumigatus*

Table2. Cost of indigenously designed Bench-Top bioreactor

Vessel	Total Volume	10 L	Cost- $ 140	Total Cost- $165
	Working Volume	07L		
	Head Plate	06 port for Thermostat, Aeration, Sampling, Air outlet, pH and Inoculum inlet ports		
	Vessel	Single wall (Borosil aspirator)		
Aeration	Flow rate	5 LPM	Cost- $ 5	
	Inlet Filter	Layer of glass wool		
Temperature Maintenance	Thermostat	20-34°C	Cost- $ 7	
pH maintenance	Digital pH meter	Hanna HI 96107	Cost- $ 20	
	Accuracy	±0.1		
	Calibration method	Manually adjusted (4,7), (4,7,10) and (7,10)		
Hollow Tubes	Six sterilized hollow tubes	Plastic tubes	Cost- $ 2	
Others	Sealing Material	Rubber cork, Silicon grease, Parafilm	Cost- $ 1	

Source: www.alibaba.com

CONCLUSION

The PPME collected from Star Pulp and paper Mill Ltd. Saharanpur (Uttar Pradesh, India) contained a high concentration of color, COD and lignin content showing its organic nature.

An indigenously designed bench-top bioreactor showed that strain of *P. chrysosporium* was more effective in removal of color, COD and lignin content from the PPME in comparison to *A. fumigatus* at pH 5±0.2, temperature 25±1°C and biomass (5% v/v). The kinetic study signifies good fit of pseudo first order Kinetic model for removal of color, COD and lignin by both the fungal strains. Significant reduction in color, COD and lignin content was achieved in our bioreactor at a fraction of the cost effective having less manufacturing and operational cost, easy to be maintained and provided good condition for biomass growth in comparison to bioreactors available in the market for PPME treatment and culture cultivation.

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